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Genetic polymorphism of endothelial nitric oxide synthase in coronary artery disease

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Background

Coronary artery disease (CAD) is a leading cause of mortality and morbidity in the Iranian population. Interaction between genetic and environmental factors determines susceptibility of an individual to develop CAD. Nitric oxide (NO) is an important endogenous vasodilator that is produced by endothelial nitric oxide synthase (eNOS) from L-arginine in endothelial and plays a critical role in the regulation of cardiovascular homeostasis.

Objective

The purpose of this study was to analyze the eNOS C786T polymorphism in CAD.

Materials and Methods

The study included 213 patients and 106 controls. eNOS rs41322052 polymorphism was genotyped using PCR-RFLP protocol.

Results

Previous eNOS T786C polymorphism studies suggested this polymorphism has an important role in cardiovascular disorders and especially in its association with the risk of CAD. We determined the prevalence of eNOS T786C polymorphism in healthy volunteers from an Iranian population and in patients suffering from CAD. Distribution

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of genotypes CC was around 100% for patients and control groups, so the C allele was not the susceptible allele for CAD subjects in this study.

Conclusion

According to the current study, there were no significant differences in endothelial nitric oxide synthase gene C786T polymorphism between healthy volunteers and patients with CAD. Therefore, genetic variation in eNOS may not contribute to etiology and risk of CAD.

Key words

Coronary artery disease, endothelial nitric oxide synthase, Iranian patients

Introduction

Coronary artery disease (CAD) is one of the leading causes of death worldwide. Many epidemiological studies show that both environmental and genetic factors have influence, but genetic factors play a more important role in susceptibility to CAD [2,1].

It is clear that endothelial cells play a critical role in the progression and clinical manifestation of the atherosclerotic process [3,4]. One of the most important products of endothelial cells is nitric oxide (NO), a major mediator of endothelium dependent vasodilation made in the endothelial cells from L-arginine through the action of the homodimeric enzyme endothelial nitric oxide synthase (eNOS). In addition to vasodilation, NO inhibits platelet aggregation, proliferation of vascular smooth muscle cells, and leukocyte adhesion to endothelial cells [5,6]. eNOS may have an atheroprotective role by these functions [7].

Nitric oxide production can be influenced by polymorphisms of the eNOS gene. The gene is located on chromosome 7q35–36 [8]. The eNOS gene is expressed and functionally regulated through multiple regulatory steps [9,10] and also by several polymorphisms [11].

The substitution of T to C nucleotide at position 786 in the 5' flanking region of eNOS gene, leading to reduce of promoter activity of this gene, is associated with CAD [12].

The purpose of the present study was to assess the association of genetic variants of eNOS 786C>T (rs41322052) polymorphism with the risk of CAD.

Materials and methods

A total of 319 subjects including 213 patients with CAD and 106 controls participated in this study.

The inclusion criteria for the patients were: (1) age at the time of CAD diagnosis: 55 years or younger in men and 65 years or younger in women; and, (2) at least 50% stenosis in a major coronary artery, or one of their branches, as determined by angiography. The extent of the disease was defined according to the number of arteries with a minimum of 50% stenosis, whether in a single vessel or in multiple vessels. Diagnosis of myocardial infarction (MI) was confirmed through patients' records using the *World Health Organization* (WHO) criteria [13] based on symptoms, elevation in cardiac enzymes or electrocardiographic changes.

All patients provided information about coronary risk factors such as diabetes mellitus, hypertension, hypercholesterolemia and cigarette smoking. Triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were measured by conventional methods of clinical chemistry. Hypertension was defined as systolic blood pressure equal to or greater than 140 mmHg and/or diastolic blood pressure equal to or greater than 90 mmHg on more than one occasion. Patients with a history of diabetes or basal glycaemia higher than 120 mg/dL were defined as diabetics.

Genotyping

Genomic DNA was extracted from 10 ml of EDTA anticoagulated peripheral blood leucocytes using salting out method.

Screening for the eNOS 786C>T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The primers used were 5'-TGGAGAGTGCTGGTGTACCCCA -3' (forward) and 5'-GCCTCCACCCCCACCCTGTC -3' (reverse).

DNA is amplified for 40 cycles, each cycle comprising denaturation at 94 °C for 1 min, annealing at 62 °C for 1 min, extension at 70 °C for 1 min with final extension time of 5 min at 70 °C. The initial denaturation stage was carried out at 95 °C for 7 min. PCR products were digested with the restriction enzyme BsmAI at 37 °C overnight. In the presence of C

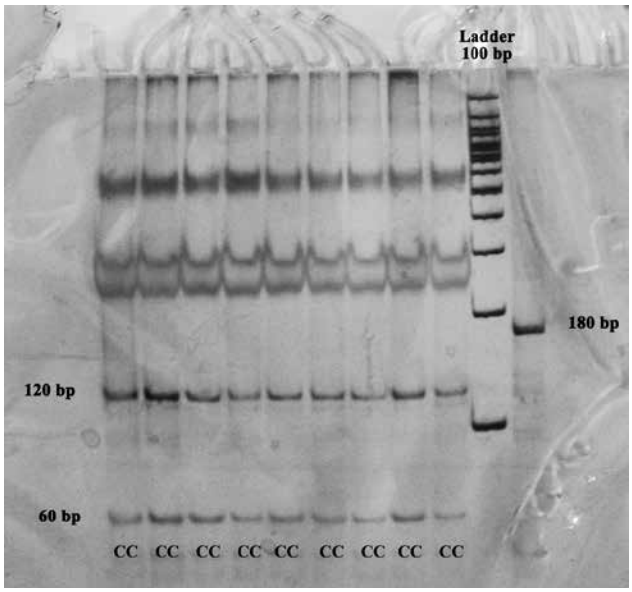

Figure 1. Representative screening for the endothelial nitric oxide synthase C786T polymorphism; 180-bp band, genotype TT; 120 & 60-bp band, genotype CC; 180, 120 & 60-bp bands, genotype CT

at nucleotide 786, the 180 base pair (bp) PCR product is cleaved into two fragments of 120 and 60 bp. The PCR products are separated on 8% acrylamid gel (Figure 1).

The validity of this PCR-RFLP analysis was confirmed by direct sequencing of several PCR samples with each genotype (Figure 2).

Results

The CAD patient group had a higher prevalence of hypertension, diabetes, smoking and family history of premature CAD compared with the controls. The patients also had higher body mass index (BMI), total cholesterol, LDL and triglycerides levels. According to our results, family history, hypertension, diabetes, smoking, obesity, high total cholesterol, LDL and triglycerides levels and low HDL significantly increased risk of CAD.

Distribution of CC genotypes was around 100% between patients and control groups.

Discussion

Recently, several studies revealed that there were various mutations on eNOS gene and these mutations might be a risk factor for CAD, MI, and hypertension. They also found that these polymorphisms differed largely among races due to large differences in the linkage pattern of –786T/C, Glu298Asp, and 4a/4b polymorphisms of eNOS among races. In this study, we investigated the relationship between T786C mutation of eNOS gene and CAD for first time in the Iranian population.

Our results demonstrated no association between C allele and CAD in the Iranian population.

A study carried out in Caucasian patients reported that eNOS (T786C) gene polymorphism is a major risk factor for CAD in this population [14]. Masafumi Nakayama *et al*. showed that an association between this gene polymorphism reduces the endothelial NO synthesis and predisposes the patients with the mutation to coronary spasm in a Japanese population [12].

Çiftçi *et al*. examination of a Turkish population indicated significantly high frequency of eNOS -786C/C genotype in acute coronary syndrome (ACS) patients than in those of controls that indicated genotype association between eNOS (786C/C) with ACS. In addition, the finding of significantly high frequency of T/T genotype in the coronary heart disease (CHD) group may support the relationship of CC genotype with ACS without CHD [15].

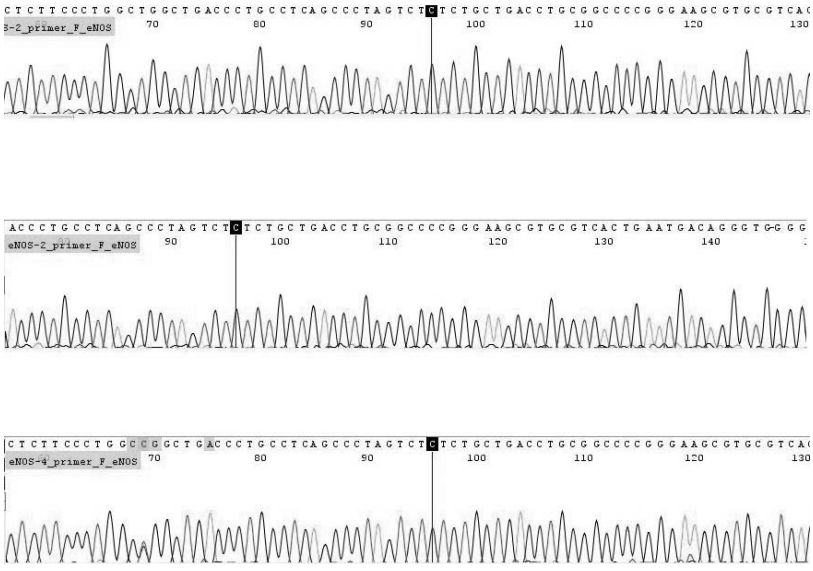

Figure 2. A sample of sequencing result for confirming eNOS genotyping. The vertical blue line indicates the position of C786T SNP

There are no more studies on this polymorphism in other populations. Future studies should seek more single nucleotide polymorphisms (SNPs) in our population and create a panel of SNPs which can be used as genetic risk markers.

In the present study, the eNOS C786T polymorphism seems to have no significant association with the risk of CAD in our patients.

Acknowledgment

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Conflict of interest: None declared

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